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FOUR HUMAN CASES OF INFECTION WITH VENEZUELAN EQUINE ENCEPHALITIS VIRUS

Following is the translation of an article by Felix Carpio, Manfred Mussgay and Albo Saturno published in ACTA Cientifica Venezolana, vol. 13- 1962. Translation by Bernice MacDonald.

The Venezuelan equine encephalitis (VEE) is a viriasis described for the first time in Venezuela in 1938 (1) in a severe epedemic among horses and mules also when the virus was isolated for the first time. Previously other cases were discovered in Trinidad (2,3,4), Columbia (5), Brazil (6) and other South American countries.

The first humanncases were reported by Casals, Curnen abd Thomas in 1943 (7), but the epédemiologic circumstances in which the infection occurred are not resolved.

No general agreement exsists as to the natural reservoir of the illness. On epedemiological bases the Mansonia titillans (Walker) and the Aedes serratu seem to be the most important vectors (6,8).

Nevertheless, approximately 80% of the cases described and reported to date, the majority have occurred in personnel who work with the equine encephalitis virus in the laboratory.

The authors are agreed on accepting the inspiration of the virus as a mechanism of infection (9) as the virus was isolated from pharyngeal washings

done during the first days of illness; although it is considered that the direct way of infection is possible by contact, it has not been described.

Four human cases of Venezuelan equine encephalitis are evident, which were contracted by exposure to the virus during laboratory work.

Causistics

Mode of infection.- In cases one and two the infection occurred in the following manner. The concentrate of virus purified with phosphotungstic acid were mixed and sprinkled on grids for investigation under the electron microscope; this procedure was effected under a plastic bell. It is believed that after removing the bell there still remained in the air some virus which caused the infection through respiratory means in the first cases. The same means is presumed in the fourth case since the person passed through the area half an hour later where the infected chicken embryos were being homogenized in a covered Virtis apparatus. In case #3 no explanation can be given as to how the infection occurred.

Clinical Observations

Case #1.- Patient 49 years old, sex-male. From 40 to 48 hours after contagion the clinical picture appeared in a violent way, beginning with an intense cephalagia of a gravative fronto-occipital type with the apin localixed in the back and legs, general

indisposition and a sensation of severe astenia, temperature 38.5°C. This picture was accompanied on two occassions with nausea and vomiting of bilious type. The clinical examination in this case reveals that the patient cooperated well in the questioning and the clinical examination, complaining of an intense cephalagia and pain in the back side of the lower limbs. The pupils respond well to light stimuli, there is pain in the eyeballs upon palpitation as well as when the eyes are moved to the extreme positions. Nose and ears without lesions. Tongue saburral, pharynx without lesions. There is pain in the cervical area upon lateral movements of extension and flexion; the pain radiates to the back upon shaking the head affirmatively. Thorax: normal configuration. Lungs: semiologically without lesion. Heart: normal sounds, rhythm 104 regular. Liver and spleen: within normal limits. Limbs: Lasegue movement positive, pain is very intense on doing the movements. Reflexes: asymmetry in answer to the osteotendinous reflexes of the lower right limb where are found more exaltadose reflexes, there is no babinsky, no clonus.

For exploration, approximately four hours after the beginning of symtomatology the temperature had risen to 39.8°C.

Second day: the temperature is 39.6°C in the morning and 39.7°C in the afternoon. Clinically

there are no modifications. Weakness is emphasized, the anorexia is greater and the patient complains of hyposmia and aguesia. In spite of the temperature the pulse decreases to 89 pulsations per minute.

Third day: the temperature is 38.5°C in the morning and 39°C in the afternoon. The picture remains almost without modifications. The cephalagia improves relatively; feces and urine normal. Pulse 80 per minute.

Fourth day: clinical picture begins to modify. The cephalagia improves notably, as well as the pain in the eyeballs, the anorexia begins to diminish, the hyposmia is no longer as intense and gustative sensations appear. Temperature in the morning 37.6°C and 37.8°C in the afternoon. Pulse 80 per minute. The patient complains of extreme weakness, diffused abdominal pain of a muscular type especially in movements of lateralization of the trunk

Fifth day: clinical improvement is substantial. Temperature in the morning 37.7°C and in the afternoon 37.2°C. Apetite is completely recovered as well as smell and taste. Feces and urine normal. The clinical examination reveals blood pressure of 120/70, pulse regular at 68, there is no cephalagia, no pain in the neck on extension or flexion of the head nor on the fist-percussion of the column. The asymmetry of the osteotendinous reflexes of the

lower limbs persists with an increase of the right side.

Sixth day; the patient is released to continue ambulatory treatment. The aforementioned reflexes returned to normal slowly.

Case#2.- Patient 32 years old, sex-male. Between 40 and 48 hours after contafion there appears in sudden and violent fashion the clinical picture characterized by intense gravative fronto-occipital cephalagia, general indisposition, fever, severe astenia, severe pain on the back sides of both lower extremeties. The physical examination reveals a prostrate patient who complains of intense cephalagia. On exploration a horixontal nystagmus of small oscillations is evident. The rest of the examination of head and neck reveals they are without apparent lesion. Thorax: lungs normal; heart: normal sounds, rhythm 102 regular; blood pressure: 130/70. Abdomen: without apparent lesions. Reflexes: normal, there is no pain on executing the Lasegue movement. There are no signs of menengism. Temperature: 39.8°C

Second day: clinically without modification. The cephalagia persists intense as well as the atenia and anorexia. Urine: urination once, normal. Temperature 38.7°C in the morning and 39.8°C in the afternoon.

Third day: clinically without modifications.

Temperature is 38.6°C in the morning and 39.1°C in the afternoon. The anorexia persists; cephalagia has improved slightly, as well as the radiculalgia. During the course of the day the patient began to feel a sensation of weight in the lower abdomen with pain and impossibility to urinate. The questioning reveals that the patient has not urinated in 12 hours and the examination reveals a painful vesical lump. A clinical diagnosos of vesical paresis and before realizing catheterism the patient is told to get up and exercise. One hour later spontaneous diuresis of 275 cc. is achieved, which notably improves the condition of the patient.

Fourth day; temperature in the morning 39°C. The patient complains of a sore throat. The cephalagia is more intense starting the night before, as well as the pain in the eyeballs. The nystagmus remains without change. The physical examination reveals hyperemic pharynx and a small left submaxillar adenopathy. The difficulty to urinate persists with a sensation of weakness and lack of strength to to expel urine. Almost permanent pain in the hypogastrium. The physical examination does not reveal a lump but on plapitation there is pain in the lower abdomen.

Fifth day; temperature in the morning 38.5°C.

Clinical symtomatology without modification. Sore throat continues the same and examination reveals no change. Temperature in the afternoon is 39°C. The diuresis has continued normal in quantitly but with great difficulty urinating.

Seventh day: substantial improvement continues. The patient is kept in bed until the tenth day and the clinical picture evolves satisfacorily with the disappearance of the cephalagia, the nystagmus, the radicula;gia and the temperature which returns to normal on the tenth day when he is released to continue ambulatory treatment.

In both cases the therapeutics were for the most part symtomatic, administering gamma-globulin and analgesics: acetosalecetic acid, codine 0.$ g. and diperone. In case #2 on the fifth day treatment with tetracicleне 100 mg. intramuscular every 12 hours was started for concomitant pharyngitis. The treatment was continued until the patient was released.

Case #3: Patient 36 years old, sex-female. There is no concrete data on the moment of contamination as the patient was working in the laboratory with material that could have caused the contagion for approximately one week. The symtomatology begins suddenly with intense frontal cephalagia and fever which by the end of the third day had

reached 38.7°C. The clinical examination done at the moment reveals a patient in good physical condition who complains of cephalagia, astenia, and general indisposition. During the first days of her illness the patient thinking that is was a simple case of gripe took antigripe medication with a base of quinine and diperone which possibly impeded the rise in temperature. The physical examination reveals: head: without data of clinical importance. Neck: without lesion. Thorax: lungs normal; heart: normal sounds, rhythm 78 regular. Temperature at the time of examination: 38°C. Abdomen: without data of clinical importance. Reflexes: normal. The syndomic picture of a feverish cephalagic was determined as an encephalitos taking into account that the patient works with infectious material.

Second day: temperature in the morning is 37.5°C and in the afternoon is 38.7°C.

It must be noted that during the entire evolution when the temperature reached 39°C an antipyretic was administered parenterally as the rise in temperature provoked at all times an increase in cephalagia, being at times intolerable for the patient. So, the maximum temperature always fluctuates between 38.7°C and 39.4°C at which time diperone was injected for the reasons mentioned.

During the whole evolution the morning temperature was always below 37.5°C. The clinical picture remained almost unmodified for 6 days.

Seventh day: the picture began to improve, the cephalagia diminished. The temperature remained normal from this moment on.

Laboratory tests: the second day of evolution a blood sample was taken for inniculation of laboratory animals. The hematological test done the same day show the following results: hemoglobin 16.2%; hematocrit; 47%; red blood cells 4,940.000; white blood cells, 3,800; bastones (canes) 3; neutrophiles, 28; lymphophiles 63; lymphocytes 29; monocytes 24; eosinophiles, 1; basophiles 1. The following day: leucocytes 3,400; bastones (canes) 1; neutrophiles 63; lymphocytes 29; monocytes 7. Two days later: white blood cells 2,500; bastones (canes) 6; neutrophiles 31; lymphocytes 44; monocytes 17; eosinophiles 2. Posterior controls show ten days later: leucocytes 7,200; neutrophies 66; eosinophiles 2; lymphocytes 23; monocytes 9; fifteen days later: leucocytes 10,200; neutrophiles 65; eosinophiles 1; lymphocytes 28; monocytes 6.

Case #4.- Patient 33 years old, sex-male. Forty hours after contagion he experiences a severe chill with a rise in temperature and general indisposition. Oral temperature was 39°C. The clinical examination reveals in this case a patient with intact sensory perception and which cooperates well in the questioning and clinical examination. The semiologic examination of the head was normal excluding a slight

cephalagia and a saburral tongue. The neck and thorax also normal. Heart with normal sounds and a rhythm of 104 beats per minute regular. Abdomen without lesion. Reflexes normal other maneuvers negative.

The clinical picture evolves in this manner: 12 hours later, beginning of symtomatology: oral temperature 39.4°C with a pulse rate of 110. Pains appear, principally articular, anorexia, gustative modifications and nausea. The patient complains of restlessness, surely feverish, and relative insomnia.

Second day: the picture is practically the same. The oral temperature is 39.5°C and the pulse rate of 106 rhythmic. A blood sample taken for innoculation of laboratory animals and a therapy exclusively analgesic and antiperitic is renewed.

Third day: the picture remains more or less the same with slight improvement and lessening of fever. The anorexia and weakness are more emphasized.

Fourth day: general condition in frank improvement.

Fifth day: ocular pains appear in lateralization movements principally and the temperature tends to go up in the afternoon. A therapy based on gamma globulin, sedative and antiperetic is used.

Sixth day: general condition improving, the temperature begins to drop and the clinical picture

changes to progressive recuperation. The blood sample taken for laboratory tests gave the following results: with the exception of leucopenia with 2,870 white blood cells per mm., with relative lympho-monocytosis, the hematic picture is normal. All other chemical tests of the blood are also normal. All other chemical tests of the blood are also normal, with the exception of 400 miligrams of cholesterol.

Seventh day: feverish temperature. Blood pressure 120/80. General condition good, recuperation of apetite and taste. Weakness persists. Leucocintal content: 4,000 white blood cells with a tendency toward the normal formula. Therapy: gamma-globulin.

Eighth day: patient is released and excepting the weakness characteristic of the viriosos, general condition is good. It is to be observed that on the tenth day the white blood count returns to normal and on the twelfth day the cholesterol descends to 175 mg.

Epicrisis

In the four cases presented the illness was benign and evolved without continuation.

The evolution was faster in cases 1, 2 and 4 than in the third case as in this one the evolution was slower and the process itself less sharp.

Virologic studies

To isolate the virus suckling rats and tissue cultures of chicken embryos were used. One day old rats were injected intracerebrally with the serum of blood samples taken 36 hours (cases 1 and 3), 48 hours (case 3) and 32 hours (case 4) after the beginning of the illness. The method used to titer the infectiousness of the serum in the celular cultures of the chicken embryos is described in another place (10). The titer of infectiousness were of 5.10^2 units formers of plates (UFP)/ccm., and 5.10^3 UFP/ccm. in cases 1 and 2 respectively. In suckling rats the titers were 5.10^3 and 6.10^4 units LD_{50}/ml. in cases 1 and 2 respectively. The blood samples of 3 and 4 were used only to isolate the virus and identify it without determining titers.

In order to identify the agents isolted proofs for inhibition of plates and of neutralization were used. A detailed description of the method used for the proof of inhibition is used in another place (10). For the proof of neutralization material from the first cerebral passage in suckling rats was used and diluted in powers of 10. Each dilution was 1:10 and normal rat serum respectively. The mixtures were kept in water baths at $37^{\circ}C$ for 30 minutes. Each mixture was innoculated intracerebrally to 5 six week old rats. Of the titers LD_{50} of both

groups (immune and normal serum) the index of neutralization of the immune serum was calculated. For the 4 isolations the index of neutralization of the serum immune to VEE was 10^4 indicating that the virus isolated was VEE. The proof of inhibition of plates confirmed this result. The increase of VEE antibodies was determined by the proof of hemo-agglutination inhibition of Clark and Casals (11). Table I shows the increase of VEE antibodies of the hemo-agglutination.

Table I

In cases 1,2 and 3 the increase of antibodies was almost the same. Eight months after infection the antibodies (cases 1 and 2) could still be detected.

Discussions

In the four cases of human infection by VEE virus the most important clinical characteristics were the sudden start of the sickness, the radiculitis and the feverish cephalea.

The difference in the clinical course in the third chase is perhaps related to an atenuation of the virus due to repeated passages in tissue cultures with it, andqin which the patient worked. This is held only as an hypothesis.

The leucopenia observed in the 3rd and 4th cases is dye probably to the great affinity between

VEE virus and lymphatic tissue and the henatopoietic organs. Unfortunately no hematologic examinations were done in the first two cases. That such an affinity exsists was demonstrated in infected acures with an attenuated stock of VEE virus (12). It is interesting to note that in infectious equine encephalitis, east or west type (EEE and WEE) leucocitosis occurs.

Man of the people in contact with the patients contracted the disease.

Table I

Increase of antibodies of VEE inhibitors of hemo-agglutination.

	Case #1	Case #2	Case #3
Before infection	1:20	1:20	1:20
3 weeks after infection	1:640	1:320	(*)
4 weeks after infection	--	--	1:320
8 months after infection	1:80	1:80	--

(*) was not tested

Summary

The clinical observations of four human cases of infection with the Venezuelan equine encephalitis virus are described. The infections occurred in the laboratory. Venezuelan equine encephalitis

virus was isolted from all four patients.

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